Marinomed

# Marinosolv®-Enabled Estradiol as Novel Treatment for Menopausal and Postmenopausal Symptoms

Whitepaper July 2024

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## Hormonal shifts: Menopause insights and treatment options - an overview

The menopause is part of every woman's life. It takes place mostly at ages between 45 and 52 years, and is initiated by a change in the hormonal status [1]. The entire process is divided into three stages: perimenopause, which can start already eight to ten years before menopause; menopause, the phase when the menstrual period stopped for at least twelve months; and post-menopause, the stage after the menopause [2].

More than 85% of women suffer during all stages from different symptoms in varying degrees associated with fluctuating and declining estrogen and progesterone levels, such as hypertension, hot flashes, night sweats and vaginal dryness, but also non-specific complaints such as sleeping problems, headache, fatigue, mood changes and loss of concentration or dry eye diseases [2, 3].

Over the last few decades, menopausal hormone replacement treatment (HRT) has been developed and today, there are several treatment options available, including different formulation forms as well as application via local and systemic routes, with their different risk and benefit profiles [4].

Recent studies on the Marinosolv® technology have explored its solubility- and stability-enhancing properties, alongside assessments of permeability and effectiveness compared to other solubility-enhancing methods and newer solubilizing agents (refer to Whitepaper: Marinosolv® - A Novel Approach to Increase the Solubility and Improve the Bioavailability of Promising Low-Soluble Drugs, March 2024).

This paper provides a summary of enhancing the solubility of estradiol, with the intention to present a novel strategy for the treatment of menopausal and postmenopausal symptoms.

## Marinosolv® as formulation technology for hardly soluble, hydrophobic compounds

Marinosolv® significantly improves the solubilization and bioavailability of hydrophobic small molecules and peptides, making it particularly effective for BCS (Biopharmaceutics Classification System) class II and IV substances. This technology is extremely useful in the early phases of drug development, where it is crucial to account for the distinct physicochemical characteristics of each compound. The principal ingredients of Marinosolv® include saponins, such as Escin and Glycyrrhizin, which are used to enhance solubility. These are combined with co-solvents and stability enhancers like Dexpanthenol to create an effective solubility-enhancing environment (refer to Figure 1). Each formulation within the Marinosolv® matrix is individually tailored to maximize effectiveness based on the specific medical indication and method of application. The focus of this technology is on liquid and semi-solid formulations, such as converting APIs previously available only as suspensions into aqueous solutions. Marinosolv® offers innovative applications for poorly soluble substances in delicate target tissues, achieving high local effectiveness with minimal systemic side effects.

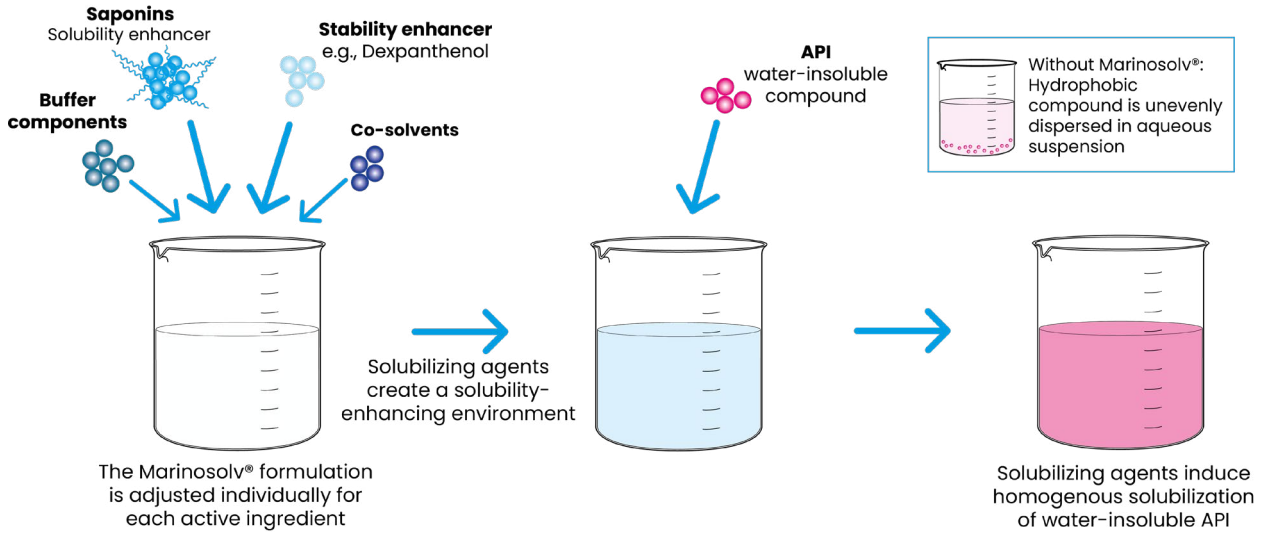



Figure 1: Marinosolv® formulation & solubilization of water-insoluble API

## Labeled estradiol as model compound to demonstrate permeation efficiency of different formulations

Estradiol (17-β-estradiol) is a naturally occurring form of estrogen, used as oral treatment for hormonal contraception and as oral or transdermal hormone replacement therapy (HRT) for the treatment of menopausal and postmenopausal symptoms [5]. It has a low bioavailability due to its very low reported water solubility of 0.2-5.0 µg/ml [6, 7]. Therefore, in the past, several approaches to increase the solubility of estradiol have been reported, such as co-crystallization with cyclodextrins [8, 9].

Our approach was to increase the solubility of estradiol by dissolving it in a Marinosolv® formulation and to investigate the permeation efficiency of a solution compared to a suspension after topical application.

To visualize the permeation into specific tissue compartments after topical application, we used fluorescently labeled estradiol (Estradiol glow, Jena Bioscience, #PR-958) as model compound, combined with a counter-staining using DAPI, and ProLong Gold for mounting. Permeation was detected via laser scanning microscopy using a Zeiss LSM880 Airyscan (excitation wavelength 405 nm and detection wavelength 410–479 nm for DAPI; excitation wavelength 488 nm and detection wavelength 534–695 nm for Estradiol-glow).

Figure 2: Comparison of Marinosolv®- enabled solution versus suspension. Left: Marinosolv®-enabled fluorescently labeled estradiol; right: estradiol as buffered suspension

In a first study, we evaluated the visualization ability of estradiol formulations *in-vitro* on different cell lines, such as HCjE (human conjunctival cell line) and HCLE (human corneal-limbal line), since estrogen receptors are present in the human cornea as well as in the conjunctiva, which can regulate key cellular events on the ocular surface [3, 9].

Therefore, Marinosolv®-enabled estradiol and estradiol formulated as suspension in buffer (PBS; phosphate-buffered saline) at concentrations of 10µg/ml and PBS without labeled estradiol was applied and incubated for 60 minutes. Results are shown in Figure 3 for HCjE cells and in Figure 4 for HCLE cells.

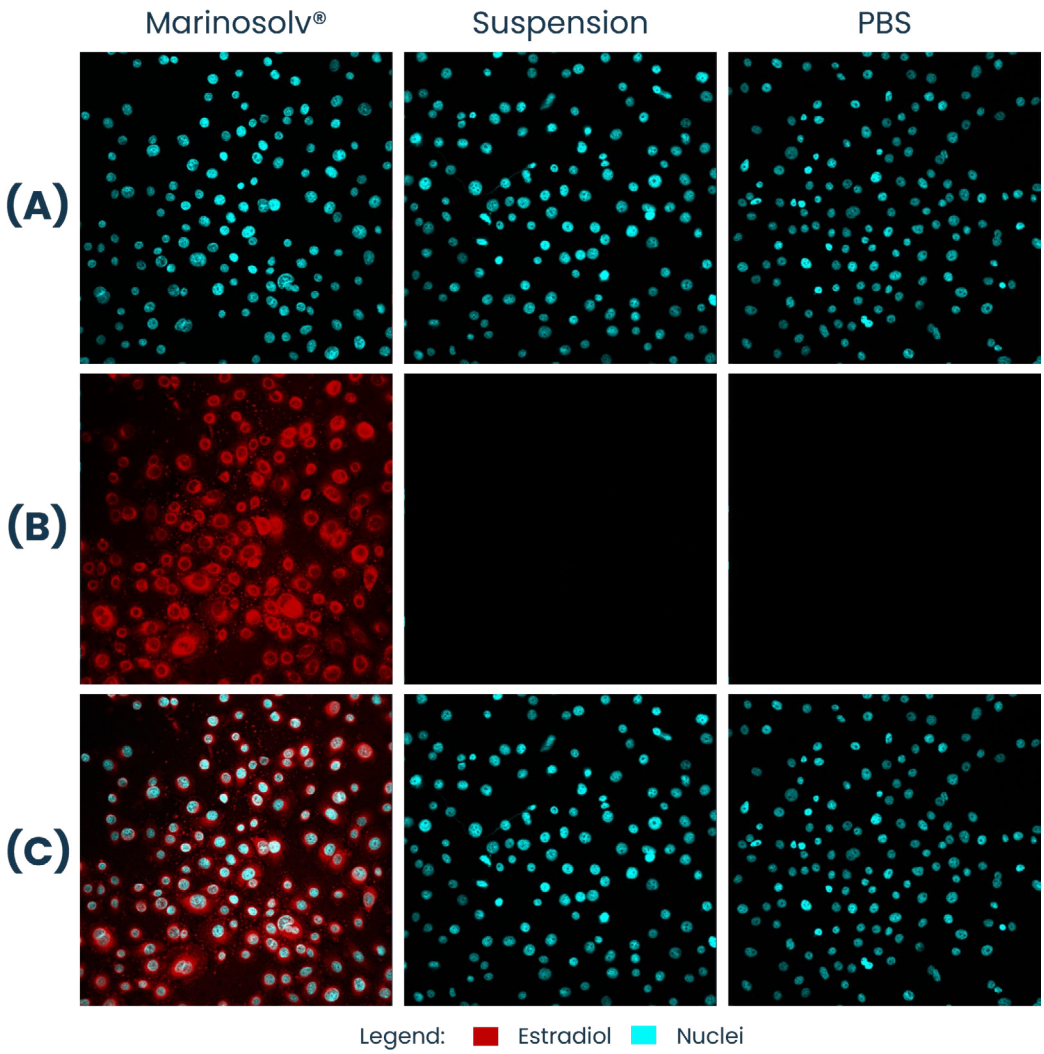



Figure 3: Labeled estradiol dissolved in Marinosolv® versus formulated as suspension and PBS without estradiol *in-vitro* on HCjE cells: (A) detection of nuclei only, (B) detection of estradiol only and (C) detection of both nuclei and estradiol

In both HCjE and HCLE cells, the DAPI staining showed a comparable amount of cells and staining efficiency in each sample. However, estradiol-stained cells could only be detected in the sample containing Marinosolv®-enabled estradiol, while all other cells remained unstained. The results indicate that dissolved estradiol permeated into these cells, while undissolved estradiol cannot permeate into cells and appears identical to the control without estradiol.

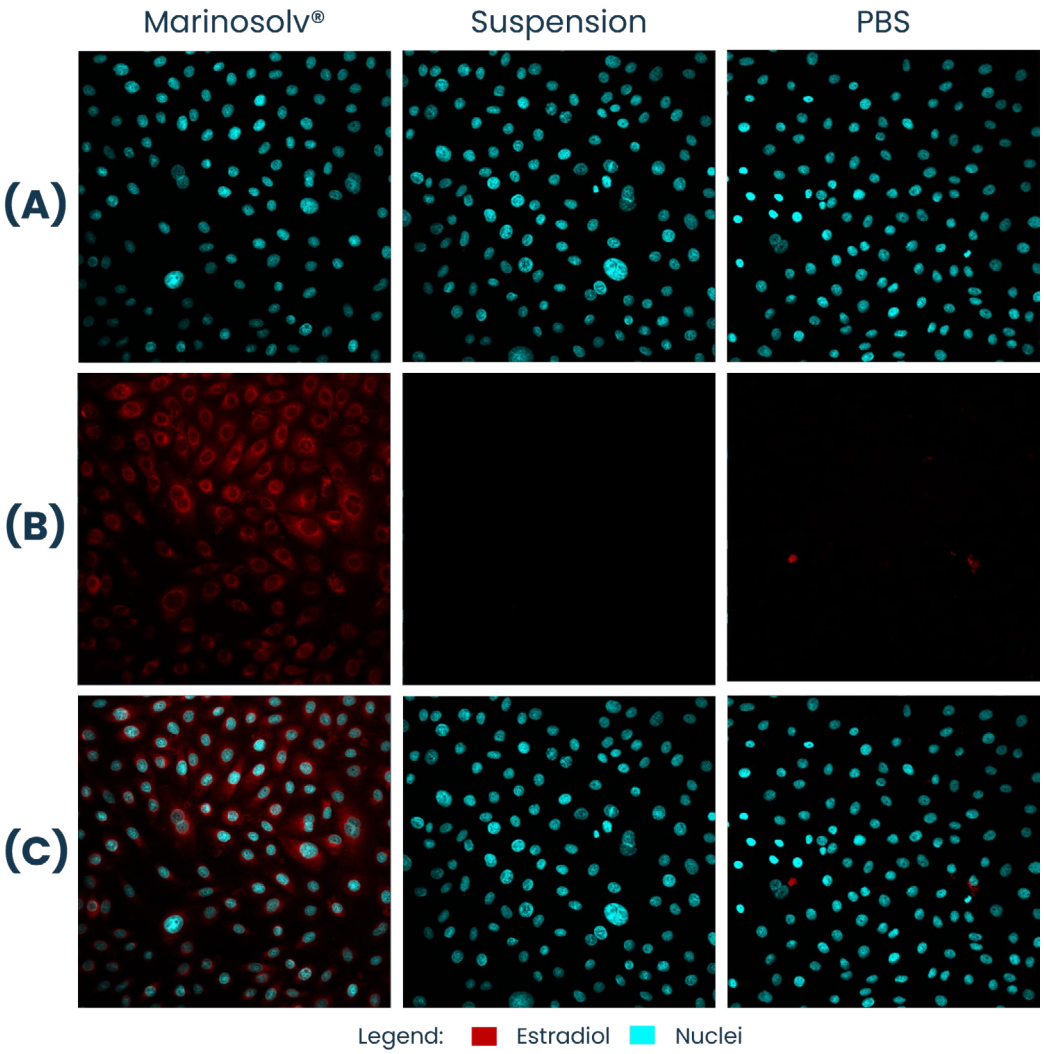



Figure 4: Labeled estradiol dissolved in Marinosolv® versus formulated as suspension and PBS without estradiol *in-vitro* on HCLE cells: (A) detection of nuclei only, (B) detection of estradiol only and (C) detection of both nuclei and estradiol

## Common treatments during meno- and postmenopause

Hormone replacement therapy (HRT) is a hormone therapy for the treatment of female menopause-associated symptoms [10, 11]. Most menopausal and postmenopausal symptoms, such as hot flashes, accelerated skin aging, vaginal dryness, decreased muscle mass, osteoporosis, sexual dysfunction, and vaginal atrophy, are caused by low levels of the female sex hormone estrogen [10, 11]. Therefore, most common HRTs are focused on the substitution of estrogens and progestogens, mostly applied systemically, orally or transdermal. Recent developments focused further on topical application via the nasal route or using eye drops, since intestinal and liver first-pass effects could be avoided.

Estradiol is often used in form of a bioidentical estrogen in HRT, although its bioavailability is low, especially when applied orally, due to its poor solubility in water [12–14]. The low bioavailability results in reduced efficacy, leading to high required doses and/or high number of required applications.

### Nasal estrogen – a novel application route for the treatment of menopausal symptoms

Due to the liver first-pass effect (which decreases the active drug's concentration upon reaching systemic circulation), the bioavailability of orally applied estrogen is limited, leading to high required dosages, thus often resulting in multiple side effects. Although the dosages for transdermal application routes are lower compared to oral forms, they are still substantial. Therefore, the intranasal route for the application of estrogen was investigated as potential treatment alternative, as it allows an improved metered, easy and rapid dosing and dose adjustment, compared to dermal treatment [15]. Clinical investigations of an aqueous nasal spray containing 17β-estradiol formulated with cyclodextrins in menopausal women with hot flashes have shown that 300 µg/day intranasal estradiol was as effective as 2 mg/day oral estradiol [16, 17]. Other studies have shown clinical equivalence of 300 µg/day intranasal estradiol compared to 50 µg/day transdermal patches [18]. In these studies, the time to reach Cmax (maximum serum concentration that a drug achieves) of estradiol was longer with both oral and transdermal estradiol compared to intranasal estradiol, hence a faster onset of action with intranasal estradiol could be shown, and the intra- and inter-patient variability was low after intranasal treatment [19]. Therapeutic efficacy of 300 µg/day intranasal estradiol has shown equivalence to 50 µg/day transdermal estradiol, and 66% women chose to rather continue treatment with intranasal estradiol than with transdermal estradiol (34%) [19]. Another treatment option is an estradiol gel for topical treatment (application of 1.25 g gel two times per day), containing 0.75 mg (1.5 mg total per day) estradiol hemihydrate each. The gel showed a low number of reported application site reactions in two clinical trials and high efficacy [20, 21]. Although the gel is dispensed by a pump that delivers metered doses, the dosing accuracy is variable due to the application on different skin areas (arm, stomach, legs), especially since an undefined amount of the gel might remain on the hands and will be washed away.

We have investigated the drug permeation of fluorescently labeled estradiol *ex-vivo* into porcine nasal mucosa at a concentration of 10 µg/ml either dissolved in Marinosolv® or formulated as suspension at similar physico-chemical conditions (e.g. pH, osmolality and viscosity). Fresh porcine nasal mucosa was obtained from euthanized pigs. Uniform parts of the nasal mucosa were taken with a 10 mm biopsy punch. Nasal mucosa pieces were placed apical side up into a 48-well cell culture plate and 50 µL per/100 mg tissue of the respective formulation was applied onto the mucosal surface. Samples were incubated for up to 60 min in a humidity chamber. After the respective incubation period, treated mucosa pieces were extensively rinsed, prepared as cryo blocks, and counter-stained with DAPI before frozen and stored at -80 °C until laser scanning microscopy.

The results, shown in Figure 5, revealed a strongly increased *ex-vivo* permeability for Marinosolv®-enabled estradiol versus a suspended formulation on porcine nasal mucosa, and a strong indication that a Marinosolv®-enabled nasal spray might increase the bioavailability of estradiol and thereby reduce the necessary dose for the treatment of menopausal symptoms.

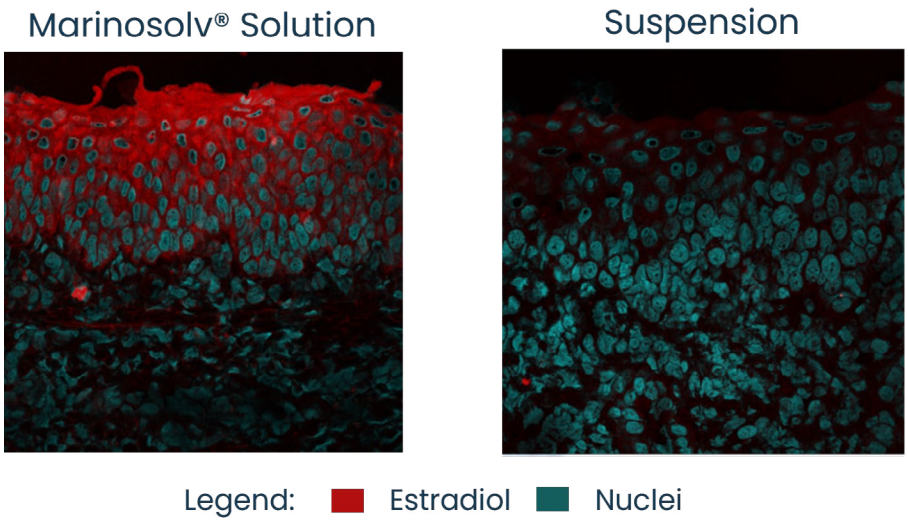
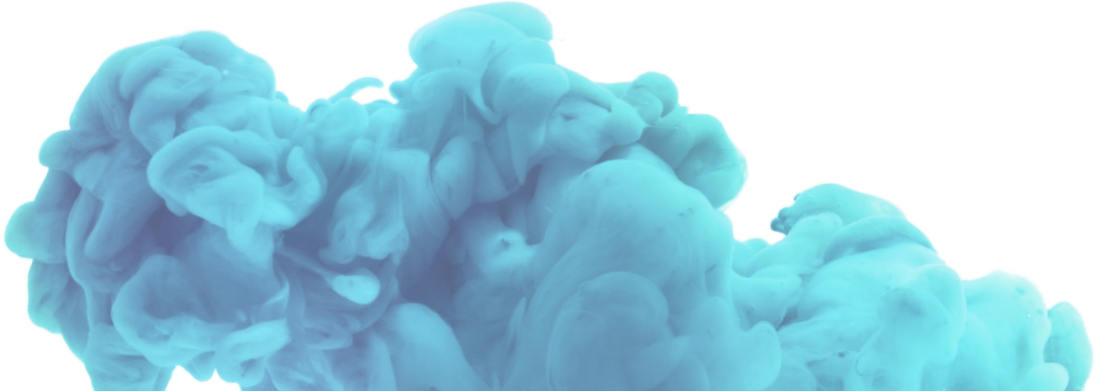



Figure 5: *Ex-vivo* treatment of labeled estradiol dissolved in Marinosolv® versus formulated as suspension on porcine nasal mucosa



## Postmenopausal eye diseases

Many postmenopausal women suffer from high intraocular pressure (IOP), the occurrence of glaucoma or dry eye disease (DED) [22]. Especially DED is a multifactorial disease characterized by tear film loss with a prevalence of DED in women over 50 years of 15.2% in 2023 [23, 24]. Therefore, several studies have suggested that HRT may be beneficial to reduce postmenopausal eye-related symptoms [25]. Other studies evaluated the efficacy of artificial tears or wetting agents as a treatment for DED [24]. Since estrogen and progesterone receptors are present in ocular surface tissues such as cornea, conjunctiva, meibomian gland, and lacrimal gland, recent investigations focused on estradiol-containing eye drops for the treatment of several diseases such as dry eye or glaucoma [3, 9, 26].

We evaluated the permeation of solubilized, fluorescently labeled estradiol in Marinosolv® compared to a suspension *in-vivo* into porcine eyes, treated four times, with 50 µg/ml of 10µg/ml estradiol, over the course of seven hours [27]. After treatment, eyes were dissected and prepared for cryo-section. For detection of fluorescently labeled estradiol in the respective compartments, cross sections of the cornea and the sclera-retina layer were prepared and counter-stained with DAPI [27]. The permeation was detected by laser scanning microscopy as described before. Permeation of estradiol into the cornea, as shown in Figure 6 (A), could be detected when dissolved in Marinosolv®, whereas treatment with estradiol formulated as suspension showed fluorescence only at the surface area of the cornea. Furthermore, in sectios of the sclera-retina-layer, shown in Figure 6 (B), estradiol could be detected only when treated with estradiol dissolved in Marinosolv®. Eyes treated with suspension showed no signs of estradiol, demonstrating that only dissolved estradiol can permeate into sensitive eye tissue compartments.

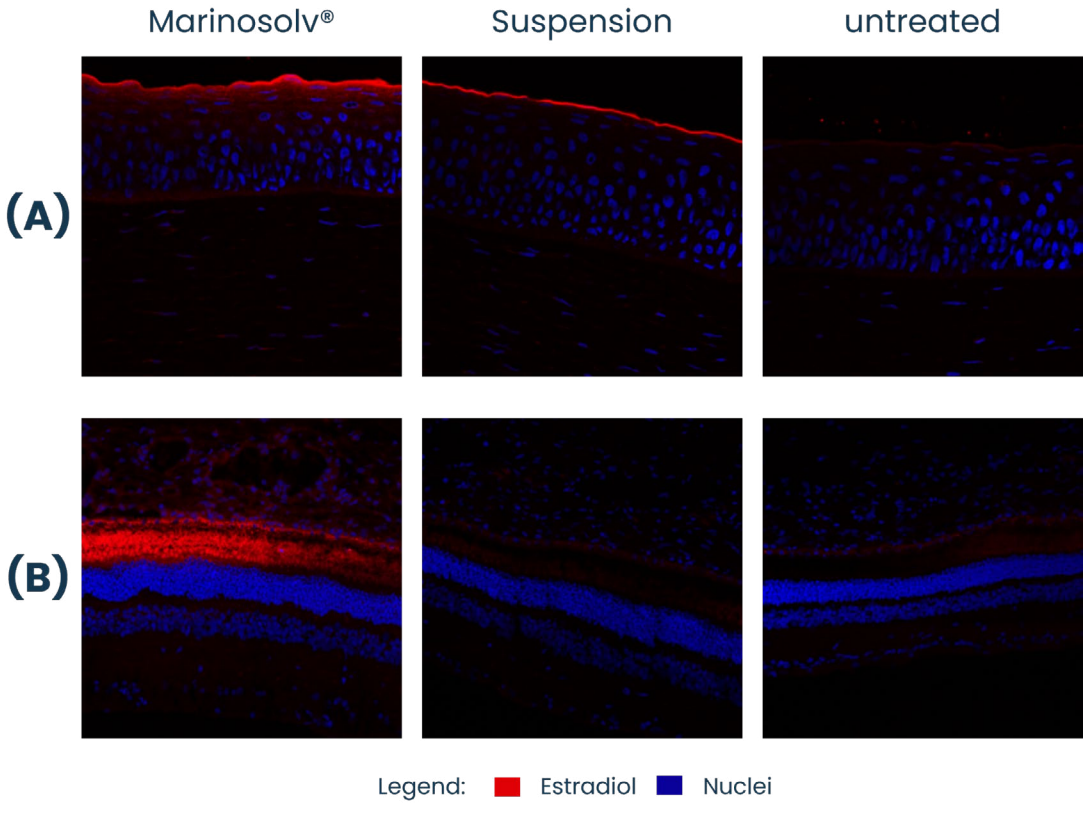



Figure 6: *In-vivo* treatment of labeled estradiol dissolved in Marinosolv® versus formulated as suspension on porcine (A) cornea and (B) conjunctiva

# Conclusion

The results summarized in these proof-of-concept studies present a novel and promising approach for the topical treatment of menopausal and postmenopausal symptoms, by increasing the solubility of estradiol in an aqueous Marinosolv® solution. Furthermore, a combination product of estradiol and progesterone can be a game changer in this field. This IP-protected formulation technology not only increases the solubility of estradiol, but also enhances the permeability into target tissues such as the nasal mucosa or eye compartments, leading to an improved bioavailability.

Based on our Marinosolv® formulation technology, several scientific papers have been published:

- **Siegl et al., European Journal of Pharmaceutics and Biopharmaceutics 134, 2019, 88-95.**
- **Nakowitsch et al., Pharmaceutics 2020, 12(9), 847.**
- **Zhang et al., Invest Ophthalmol Vis Sci. 2020; 61(1):4.**
- **Zieglmayer et al., Clin Exp Allergy. 2020; 00:1–13.**

Read here all Marinosolv®-related publications:
https://www.solv4u.com/en/news/publications.

The advantages of using a Marinosolv®-based formulation, including safety and efficacy, have been confirmed through preclinical toxicological evaluations and clinical trials, including a phase II study for ophthalmic use and a pivotal phase III study for nasal sprays. Marinomed Biotech AG not only develops its own product lines utilizing Marinosolv®, but also provides this formulation technology to third parties through its Solv4U technology partnerships. This approach facilitates a distinctive pharmaceutical development program that includes both established APIs and new chemical entities (NCEs).

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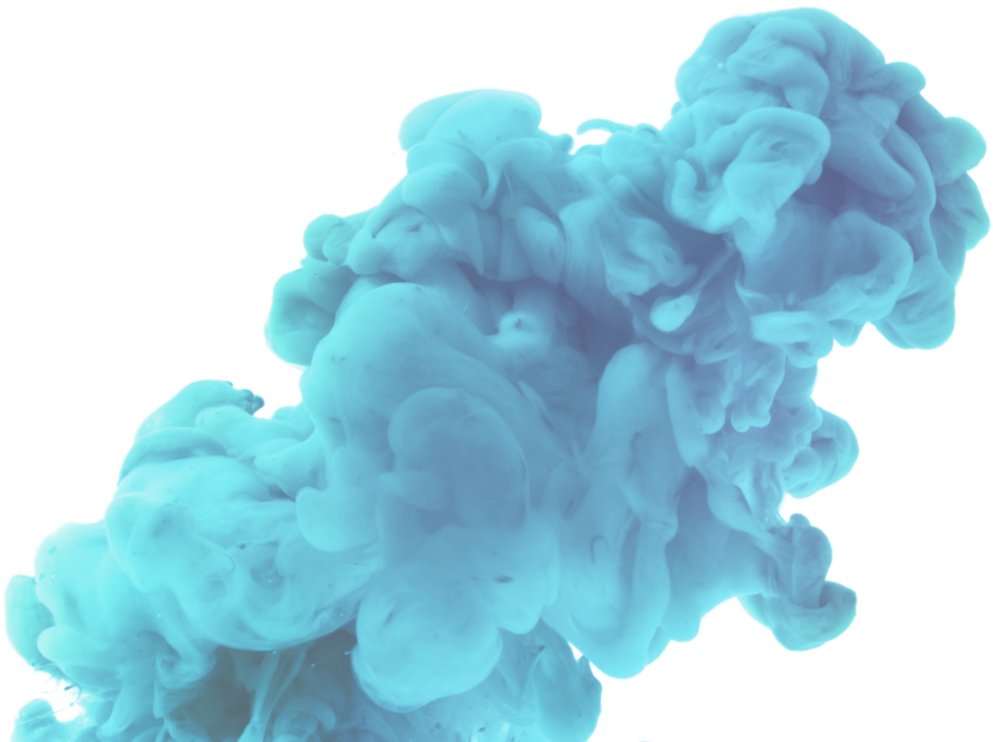

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## About Marinomed Biotech AG

**Marinomed Biotech AG** is an Austrian, science-based biotech company with globally marketed therapeutics and a growing development pipeline. The Company focuses on the development of innovative products based on two patent-protected technology platforms, Marinosolv® and Carragelose®. Marinomed is listed on the prime market segment of the Vienna Stock Exchange (VSE: MARI).

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